

# BIOLOGICAL BULLETIN

## STUDIES IN THE LIFE CYCLE OF SIMOCEPHALUS VETULUS.

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### I. INTRODUCTION.

These studies on *Simocephalus* were begun at the Hull Zoölogical Laboratory of the University of Chicago, in 1913. The life cycle is very complex and has not been worked out satis-

factorily in all of its phases. Although very creditable work has been done by Chambers ('13) on spermatogenesis in *Simoccephalus*, and by Weismann and a host of others on the natural history, oogenesis, fertilization, etc., of the Daphnians in general, there is still urgent need of more data. Much time and effort have been expended by the writer in the attempt to perfect a technique which would be adequate to the cytological problems. Considerable progress has been made with this phase of the work yet the study is far from completion. The present paper will be devoted to some problems naturally arising in the course of such studies, which should be solved before or at least in coördination with the cytological problems, else the latter would lose much of their significance.

Among such problems are the following: the normal general life cycle must be known; whether this may not be modified by altering the external conditions and if so, to what extent and how; whether the species may not express itself in all of its forms under each of several sets of conditions, or if there are certain environmental factors which bear special relations to certain forms; whether the failure to produce sexual forms during the parthenogenetic phase is due to a deficiency in the environmental complex or to internal conditions such as age or is dependent simply upon the general rate of metabolism; the normal proportions of males and females; the ratio of sexual<sup>1</sup> to asexual females, the sequence of broods; the cause of senescence of the cultures; the cause of the appearance of the ephippial eggs; the time in the ontogeny at which they appear; the precise function of these eggs, *i.e.*, whether the importance of the ephippial egg is centered in the egg as a means of tiding the species over unfavorable seasons or periods, or in the stem mother developing from it; the relation of the males to the production and development of the ephippial eggs; the normal length of the late period; if it be of definite duration; whether it can be shortened and if so, how; whether the offspring of a stem mother

<sup>1</sup> By the term "sexual female" in this paper we designate those females producing a series of ephippial eggs which require fertilization; by "asexual female" those which reproduce only parthenogenetically. All so-called sexual females are destined to pass from their sexual phase into the parthenogenetic phase and so remain.

are different from the offspring of females produced parthenogenetically; whether any difference is to be found between the offspring of parthenogenetic females which have passed through a sexual period and those which have not; whether a sexual female may not be retained in the sexual state, etc.

This paper presents data accumulated chiefly during the four years from 1914 to 1918 at Carleton College biological laboratory, Northfield, Minn., bearing upon the solution of these and other problems and definite conclusions regarding some of them. There naturally arises the very interesting and important problem of ascertaining just what correlations exist between the general course and the cytological aspects of the life cycle. The details of my work on this latter phase of the problem are to be given in another paper, now under way.

## II. LITERATURE REVIEW.

The theory that there is a sex cycle in Cladocera which is independent of external factors was formulated by August Weismann. It is now most certainly known that, although the correlation existing between environmental factors and the production of parthenogenetic and sexual forms is not exact, certain environmental complexes do exist which completely inhibit the appearance of males and sexual females.

A review of the very extended work of Weismann (1877-87) and his contemporaries such as Kurz (1875) and Schmankevitch (1877), and of later writers as Issakowitsch (1907), of Kuttner (1909), Woltereck (1909), Papanicolau (1911) and others, leaves one very unsettled as to what is the general determinative principle underlying the course of events in the normal life cycle of even the well known and much studied genera such as *Moina*, *Daphnia*, and *Simocephalus*. The Weismannian conception of a relatively fixed "generation cycle" for *Cladocera* is well known, and has been adopted wholly or in a modified form by practically all writers up to about 1914, although its truth had been questioned by several experimenters. About this time some results were obtained by several workers using various species of the three genera mentioned, which must be interpreted as positive evidence against the theory. Since Weismann's

time the recorded opinions range all the way from unconditional acceptance of his extreme view to a complete lack of faith in it. For convenience of discussion these various views may, in general, be grouped under two chief heads:

1. That external factors are of little or of no importance in relation to the succession of periods of parthenogenetic and sexual reproduction. This was the conviction of Weismann, based upon his researches extending over a long period of years. This theory was broad enough to cover all forms of life in which there is a succession of parthenogenetic and sexual reproduction. Accordingly the evidence for the theory extends over a wide field, and it has numerous supporters, having been for a long time the orthodox view.

This general view is supported by Keilhack (1906) and Ekman (1905) working on *Polyphemus*, by Popoff (1907) working on various protozoa and metazoa, and by Punnett (1906) and Whitney (1907) working on *Hydatina*, and by others. The last two authors concluded that the age of the strains is a weighty factor in causing the appearance of the sexual forms. The conclusions of Strohl (1908) constitute a clean cut statement of the views up to that time. Working with *Polyphemus*, he concluded that there was no reason for abandoning the well grounded views of Weismann. Kuttner (1909) working on *Simocephalus vetulus* and other Daphnians adopted Weismann's extreme view. McClendon (1910) considers that "The life cycle of a Daphnid is, therefore an heredity tendency, but can be influenced by nutrition and probably by temperature and the accumulations of excretions," and he adds "Nutrition is the most important factor."

The extended experiments of Woltereck (1909-11) on *Cladocera* led him to admit automaticity as one of the factors in determining the course of their life cycle. Assuming that Woltereck's cultural conditions (1911, fig. 4, p. 152) were kept the same in all four cultures for the full four years, one would be forced to the conclusion that there are periods when environmental factors are of no influence, and possibly other periods when they are, since the four cultures responded not only differently in different years, but different cultures responded differently in the same years; in other words, the conclusion would seem to follow that



there is autonomous variation in this respect, which places the whole question beyond the pale of possible experimental proof. The careful experiments of Papanicolau (1911) merit more consideration than is admissible in this paper. The views of Issakowitsch (1907) and Scharfenberg (1911) agree with those of Woltereck and Papanicolau in that they all assume certain inherent tendencies to sexuality which cannot be completely overcome by any kind of environmental conditions.

2. The alternative view is that outer causes, such as chemical substances, hunger, temperature, kind of food, etc., are largely or entirely responsible for the varying degrees of sexual and parthenogenetic reproduction. Evidence for this heterodox view had begun to accumulate at least as early as 1875, when Kurz noted a correlation between the drying up of the water and the appearance of sexual forms in *Cladocera*. Two years later Schmankewitsch (1877) gave as his opinion that the efficient cause is the increasing salt concentration due to evaporation. Among the unfavorable conditions mentioned by Kerhervé (1892, p. 236) poor nourishment is particularly emphasized as being responsible for the appearance of both males and sexual females. Ostwold (1094) found temperature singularly effective as a cause of sexuality in Daphnids. By varying the temperature he produced at the same time all of the forms that are found in nature at different seasons. Langhans (1909, p. 291) says that Weismann's theory of a fixed generation cycle will not bear critical examination.

In regard to other forms whose life cycle is in general similar to that of *Cladocera*, there is much diversity of opinion, although evidence against a fixed internal "cycle" is rapidly accumulating.

Finally we must include under this second category the very significant and definite conclusions of Grosvenor and Smith (1913) working with *Moina rectirostris*, of Banta (1914) working with *Daphnia pulex*, and of Agar (1914) working with *Simocephalus vetulus*, each of whom has conclusively demonstrated that, for the form experimented on, there are certain environmental complexes which will indefinitely inhibit the appearance of males and sexual states in any of the females. It is of interest to note that in some instances the conditions which will thus prevent the full expression of the species is quite narrowly prescribed.

A third category of opinion is sometimes tacitly implied by the manner in which the problem of the succession of forms in *Cladocera* is discussed by those authors who believe that the primary causes are cytological. Were this true the problem would not of course be thereby removed from the domain of possible influence by environmental factors. Here should be mentioned the well-known "kern-plasma" theory of Hertwig. It is supported by Papanicolau, Issakowitsch, Popoff and others.

The results of Von Scharfenberg and Papanicolau have been brought by Child (1915) into relation with certain phases of his general theory of organic constitution, as developed in his book, entitled "Senescence and Rejuvenescence." He says (p. 391): "Von Scharfenberg and Papanicolau found that a change in egg character occurred, not only in the course of successive generations, but also in the course of single generations, *i.e.*, the eggs produced early in the life of a female are more likely to develop parthenogenetically into females and those produced later in life into males or to be zygogenic winter eggs. In the earlier generations of a cycle the male producing and zygogenic eggs appear later in the life of the individual, in later generations earlier." I am convinced that the conception that zygogenic eggs normally arise in Daphnians *after* the parthenogenetic eggs is based on entirely insufficient evidence. Definite statements bearing on this point will be found in Papanicolau's papers (1910, p. 740, and 1911, p. 82). These views are in substantial agreement with those of Issakowitsch (1907) and Scharfenberg (1911), and diametrically opposed to my own since I have never found an instance of the production of ephippial eggs following a period of parthenogenetic reproduction. I am convinced that these conclusions could not have been based upon observations on isolated individual females of *Simocephalus vetulus*. Grosvenor and Smith (1913, p. 514) working on *Moina rectirostris* state: "We did not find any case of a female that had produced eggs parthenogenetically turning into an ephippial female."

For several years the writer was not able to duplicate the results of those experimenters who claimed that, under certain conditions, reproduction in some *Cladocera* will proceed for an indefinite number of generations parthenogenetically, and that

the change to sexual reproduction, together with the degeneration that so often accompanies it under experimental conditions, are wholly dependent upon the environment. Later experimentation however (experiment 5), has demonstrated their contention to be correct, at least for *Simocephalus vetulus*. Though these last experiments are of a very different type from those described by Agar (1914*b*, Table I.), Grosvenor and Smith (1913), and Banta (1914), who succeeded in carrying Daphnians through many generations with no loss of vigor, they are corroborative of their results. That the success of these authors in rearing purely parthenogenetic generations for an indefinite time could not have been due to the accidental selection of lines with a strong internal tendency to parthenogenesis is rendered certain not only by the use of several distinct lines, but also by parallel cultures subjected separately to unfavorable temperatures and crowding, which gave a large proportion of males and sexual females. The genera used were *Simocephalus*, *Moina* and *Daphnia*. Banta carried *Daphnia pulex* for 127 generations with no loss of vigor.

In groups other than Cladocera in which it has been found that an indefinite number of generations can be reared parthenogenetically, it seems that it has always been possible to bring on sexuality by a proper change in the environment, which would indicate that pure lines with respect to parthenogenesis do not exist. It now seems altogether likely that bisexuality may be indefinitely inhibited in many more of the lower animals than experimenters have been aware of heretofore. The conditions under which this is possible have been found in some instances to be narrowly circumscribed, as *e.g.*, certain Cladocera require the high temperature of 28° C., although the more recent authors seem to believe that a considerable number of species of Cladocera will be found which can be induced to reproduce by parthenogenesis indefinitely if only the external conditions are properly manipulated. It appears also that, whatever may be the explanation, any set of nearly uniform conditions finally becomes prejudicial to continued parthenogenesis. Parthenogenetic reproduction seems to be favored by conditions which are conducive to rapid growth, though they may vary within certain limits.

Each species of Cladocera found capable of pure parthenogenesis has its own specific requirements as to which environmental factors may vary and to what extent without it becoming zygogenic.

### III. MATERIAL.

On taking up this work much difficulty was encountered in rearing algæ as food for the Daphnians. Numerous formulæ were tried with varying success, but for some reason they did not thrive on algæ artificially produced. A simple and satisfactory solution of the difficulty was discovered in the following method: A number of three- or four-gallon aquaria nearly full of water are placed where they will not receive the direct rays of the sun and stocked with several kinds of unicellular algæ. One to three frogs, depending upon the size, which have been in captivity until little or nothing remains in the alimentary canal, are placed in each of the jars. Of course the aquaria must be covered, in part to retain the frogs, but the more important reason for this is to insure the maintenance of a high percentage of carbonic acid gas and the depletion of oxygen. Under such conditions the algæ grow rapidly until a condition of equilibrium is reached. When a sufficient quantity of algæ has developed a single large Daphnian with brood pouch full of eggs or embryos may be introduced and the frogs removed, or one may be left in with good results. When the Daphnians have overstocked the culture they may be strained out with a cloth and transferred to a new jar. The brown sediment of the old culture, which is excreta and dead algæ, should be carefully removed so as not to loosen the algæ on the sides of the jar, new water added and more algæ grown in the manner indicated above.

The above method has served me very satisfactorily for *Simocephalus vetulus*, though it is not so well adapted to some other forms. *Daphnia pulex* thrives in water which is more or less filled with putrescent matter. In a pond frequented daily by cattle and thus kept very roily and malodorous, I found this species in such numbers that by a single dip of the net I secured hundreds of them. For best results one needs to work out special methods for each species. In all of my work I have used *Simocephalus vetulus* unless otherwise stated.

## IV. ISOLATION EXPERIMENTS.

In order to ascertain the course of events in the normal general life cycle I isolated numerous specimens, at random at first, and kept a complete record of all of their offspring. Some of these will be presented in detail. For convenience all experiments will be referred to by number.

*Experiment 1.*—On June 23 a very large female with the brood pouch full of embryos was isolated. On June 24 a brood of 47 asexual females was extruded. These embryos were saved and a first brood secured from each of 25 of them. The remainder died early. There were 13 pure broods of males, the numbers in the broods being as follows: 4, 3, 1, 3, 1, 1, 1, 2, 2, 2, 6, 2, and 7. There were 6 mixed broods as follows: 3 males and 6 females, 8 males and 4 females, 6 males and 6 females, 1 male and 6 females, 5 males and 3 females, and 2 males and 4 females. The numbers of females in the pure broods of females were: 4, 5, 6, 3, 4 and 2. Now with this single instance before us we might well ask why this great variety in the offspring of the members of a pure brood of 47 asexual females? But in the light of further experimentation along this same line we may reasonably suppose that had the mother of these 47 female embryos lived to produce other broods, some would have consisted of males or at least would have contained males.

Another female isolated at the same time lived until July 13, when she died with embryos in her brood pouch. Her series of broods is as follows: 1 male and 6 undetermined, 7 females, 15 males, 50 females and 1 male, 15 males, 9 females, and 13 females. The 50 females of the mixed brood were saved and the first brood of each of 19 of them was secured. There were 12 broods of males containing 8, 12, 3, 8, 12, 3, 12, 4, 5, 41, 12 and 6. There was one mixed brood of 12 males and females, and 6 pure broods of females contained 6, 10, 1, 3, 2 and 6.

There seemed to be no constancy as to the ratio of males to females in the offspring of these isolated females, nor in the sequence of broods, some producing males first, some females, and others mixed broods, in a most capricious manner. Hence these miscellaneous experiments demonstrated the desirability of much more extended experimentation to discover if possible what order might underlie this apparent confusion.

*Experiment 2.*—The following experiment was designed to show in particular the normal ratio of males to females, and their sequence, though it furnishes data bearing upon several other points. It was begun on June 23 by the isolation of a single female from a laboratory culture. She produced the following broods on the dates indicated:

On June 26 a brood of 43, 27 being female, 16 not living.

On June 28 a brood of 49 females.

On July 3 a brood of 75 females.

On July 7 a brood of 7 males.

On July 10 a brood of 6 females.

On July 11 the isolated female died.

Of the brood produced on June 28, 45 which lived were saved for experiment. These were isolated and the sex of all of their offspring determined, and in several instances the kind of females *i.e.*, whether sexual or asexual. The members of this brood are numbered. The date of the first broods of each of the first 17 was July 4, of all of the remainder it was July 5. The date of the death of each female is given at the end of her series of broods. The date of the last brood is practically always not more than one day before the death of the female. The second brood of female number 1 is a mixed brood and they are so indicated throughout.

1. 9 females, 5 females and 9 males, 9 females, an undetermined brood, July 15.

2. 9 females, a mixed brood of about 20 containing many dead, 6 females, July 12.

3. 4 females, 3 males and 6 undetermined, 11 males, 4 females, July 13.

4. 4 females, 18 males and 2 females, 6 females, 1 female, July 13.

5. 3 females, 11 males and 8 females, 16 females, July 9.

6. 9 males, 3 undetermined, 6 females, 22 females, July 10.

7. 7 females, 7 females and 8 undetermined, 6 females, 5 females, an undetermined brood, July 14.

8. 6 females, 3 females and 10 undetermined, 10 males, July 7, 14.

9. 8 females, 2 males, 4 males, July 9.

10. 6 females, 3 males and 16 undetermined, July 7.
11. 6 males and 1 undetermined, 4 males and 1 female, 4 females, an undetermined brood, July 13.
12. 8 males, 3 males, 3 females, an undetermined brood, July 9.
13. 7 males, 1 male, 7 females, July 9.
14. 9 females, a mixed brood undetermined, 6 females, July 12.
15. 6 females, 9 males, 7 females, July 8.
16. 10 males, 6 males, 5 females and 4 undetermined, 10 females, July 9.
17. 9 females, 38 females, 10 males, July 15.
18. 12 females, 7 females and 6 males, 10 undetermined, 7 females, July 9. All of the last brood of 7 females were asexual but some of their offspring were sexual.
19. 6 females, 10 males and 14 undetermined, 7 females, 14 females, July 9.
20. 8 females, 21 males, 8 females, two other undetermined broods, July 10.
21. An undetermined brood, 11 males and 12 females and 14 undetermined, July 9.
22. 7 females, 12 males and 7 females, an undetermined brood, July 9.
23. 10 males, 2 males and 13 females, 3 females, July 13.
24. 4 males, 6 asexual females, 10 females, 2 sexual females, 15 males, 24 females of which 2 were sexual and 2 asexual and 8 males, (the other 20 females of the last brood died too early for their sexuality to be determined), 13 females 7 of which were asexual and 6 undetermined, 16 asexual females and 1 male, 2 females 1 being asexual and 1 sexual, 22 females 12 being sexual and 10 undetermined, the next two broods were accidentally left together but their sum was 35 females of which 12 were sexual and 11 asexual while the remaining 12 were undetermined, the final brood was 7 females of which 5 were sexual 1 asexual and 1 undetermined, August 2.
25. 5 females, 20 males, 4 females, July 9.
26. One male and 5 females, 11 males and 2 females, 7 females, July 10.



27. 4 males, 1 male and 4 females, July 7.
28. 7 females, 12 males, 3 sexual females, 7 males, 1 male, July 12.
29. 5 males, 7 males, 6 males, 2 males and 11 females, July 14.
30. 5 females, 8 males and 9 undetermined, July 8.
31. 4 males, 8 males, July 7.
32. 8 females, 16 males, 1 male, July 9.
33. 2 females, 22 males, 8 females, July 9.
34. 6 males, 7 males, July 8.
35. 6 males, 5 males, July 8.
36. 8 males, 5 males, 4 females, July 9.
37. 8 females, 7 males, July 8.
38. 5 females, 22 females, 6 males, an undetermined brood, 20 females, 1 male and 35 females, July 15.
39. 19 males, an undetermined brood, 15 females, July 8.
40. 4 females, July 7. (mother with abnormal carapace, offspring normal).
41. 3 males, 3 males, 6 females, 7 males and 7 sexual females, 5 males and 2 sexual females, 1 sexual female, 1 female and 5 undetermined, 20 females 15 of which are asexual the other 5 may be, 15 females, 17 females, 3 females 2 of which are sexual and 1 asexual, 2 females 1 of which is asexual the other undetermined, 9 females 7 of which are asexual 1 sexual and 1 undetermined, July 31.
42. 6 males, 2 males, 13 females, 1 male and 2 females, July 13.
43. 5 males and 2 females, 11 males, 7 females, 11 males, 1 male, 6 females, 11 males and 1 female, 2 males, a brood of females, 5 females, an undetermined brood, 1 female, July 26.
44. 7 males, 8 males, 6 males, 2 males, 10 females, 9 females, 24 undetermined, 9 females, 8 females, 12 females, an undetermined brood, 4 females, 4 asexual females, August 4.
45. 8 females, 23 males, an undetermined brood, 8 males, 8 males, 15 males, 10 females, 18 females, 1 male and 2 females, 1 sexual and 1 asexual, an undetermined brood, 7 females, July 24.

## SUMMARY OF EXPERIMENT 2.

The number dying without offspring . . . . .	4
" " possibly producing no males . . . . .	3
" " " " " females . . . . .	2

The percentage of males is about . . . . .	42
The number of times the first brood was purely female . . . . .	26
“ “ “ “ “ “ “ “ “ male . . . . .	16
“ “ “ “ “ “ “ “ “ a mixed brood . . . . .	2
“ “ “ “ “ “ “ “ “ undetermined . . . . .	1
“ “ “ “ “ last “ “ “ purely female . . . . .	28
“ “ “ “ “ “ “ “ “ male . . . . .	11
“ “ “ “ “ “ “ “ “ a mixed brood . . . . .	6
The total number of mixed broods . . . . .	22
The number producing no mixed broods . . . . .	25
“ “ of times the broods immediately preceding and follow- ing mixed broods are of the same sex . . . . .	9
The number of times they are of a different sex . . . . .	4
The average number in each pure first brood of females . . . . .	6.6
“ “ “ “ “ “ “ “ “ males . . . . .	7
“ “ “ “ “ mixed brood . . . . .	16
“ “ “ of offspring per individual . . . . .	39
“ “ “ broods “ “ . . . . .	4.3
“ “ “ hours between the broods . . . . .	45.3
“ “ “ female broods per individual . . . . .	2.1
“ “ “ male “ “ “ . . . . .	1.3
“ “ “ females in each female brood . . . . .	4.5
“ “ “ males “ “ male “ . . . . .	11.9
“ “ “ “ “ produced per individual . . . . .	15.5
“ “ “ females “ “ “ . . . . .	20.7
“ “ “ days each of the 45 females lived . . . . .	14
The total number of offspring was slightly over . . . . .	1795

The 45 females under consideration in this experiment were divided into three groups. The first group, consisting of 24 individuals, was kept in water taken from an "asexual" culture and were well fed; the second group of 20 were also well fed but were kept in water taken from a culture which was producing an abundance of males and sexual females; the third group of 5 being reared in the same kind of water as the second but were poorly fed. The results of this part of the experiment are given in the following table in which the data have been reduced

	Group 1	Group 2	Group 3
Average length of life in days . . . . .	12.4	11.0	27.2
“ number of broods . . . . .	3.6	3.0	10.6
“ “ “ female broods . . . . .	2.1	1.1	5.2
“ “ “ females produced . . . . .	21.6	12.3	43.4
“ “ “ males “ . . . . .	11.5	15.6	29.2
“ “ “ male broods . . . . .	.9	1.4	3.2
“ “ “ offspring . . . . .	37.6	26.6	80.6
	%	%	%
Mixed broods . . . . .	9.	10	11

to the basis of the individual, the data on the first, second, and third groups being given in the first, second, and third columns respectively, of the table.

Comparing these three groups we find that the differences when averaged are not great enough to be significant. The members of the third group lived roughly two and one half times as long as the others, and should we reduce the data to a common unit of time the correspondence would be very close. For instance, the number of broods produced by the first group per unit of time, say 27 days, would be 10 ( $2.25 \times 3.6 = 10.00$ ), while the third group produces just 10.6. The number of female broods produced by the first group would be 4.72 ( $2.25 \times 2.1 = 4.72$ ), as against 5.2 broods produced by the third group, etc. As to all of the main points under consideration the figures agree so closely that we must conclude that the kind of water in which the three groups were reared and the relative food supply had little or nothing to do with the ratio of sexes. In the general discussion of this experiment further data are given based on the observation of isolated females whose ancestry is now known for one or two generations, with conclusions regarding the relation of sexual to asexual females, males to ephippial egg production, etc. It is deemed better however, to defer this until after the presentation of another general isolation experiment in which stem mothers were used instead of females selected from the general cultures.

*Experiment 3.*—Although most of my experiments to induce ephippial eggs to hatch have been failures I have given them in detail further on in this paper because of the fact that so little has been accomplished along this line. I have succeeded in securing about 70 stem mothers and have had a fair degree of success in rearing them. All of these were isolated at once and a complete record was kept of the number of broods, the kinds of individuals in each brood, length of life, etc., as shown in the table below. It was found that a large number died early, producing few or no offspring. A few however produced as many as 9 broods. The average number of broods produced per stem mother was slightly over 6. Hence in the tabulation of results no account was taken of stem mothers whose broods were too few,

since these were probably not perfectly normal, else they would have lived longer, nor of broods beyond the sixth except to consider them in determining the average number of broods per stem mother, since the averages were not materially changed by eliminating them. Accordingly only 24 stem mothers were considered fit to include in the final averages, and for similar reasons the number of mothers in the  $F_2$  and  $F_3$  generations were reduced to 16 and 12 respectively. I was much surprised to note the large proportions of males and sexual females in the broods of these stem mothers. This observation suggested the experiment of rearing several generations to discover the relative proportions of males and sexual females in the first and later generations, to compare the offspring of the stem mothers with that of the females produced parthenogenetically, and to learn what difference, if any, is to be found between the offspring of sexual females after they have passed through the sexual state and of those females which never pass through this state. It seemed that even if passing through the sexual state did not affect the ratio of the sexes, or of asexual to sexual females, in the immediate offspring, that the effect might conceivably be cumulative and would be apparent in the succeeding generations. Hence the sexual and asexual females in the  $F_1$  generation were segregated and two distinct lines, one sexual and the other asexual, were carried to the  $F_3$  generation. Perhaps the most conspicuous feature in the records of this experiment is one shown only by the individual records of the females, namely that they are variable in the extreme. A given stem mother may produce nearly all males, or one kind of females, for several broods, or throughout her life, or they may appear combined in all proportions, just as is shown in the detailed individual records given in experiment 2, female number 41. The most important facts however are to be deduced from the summary given at the end of the tabulated results. In order to facilitate the comparison of results in the sexual and asexual lines in the second and third generations, I have placed the figures in juxtaposition and have reduced them to a percentage basis at the end of the table, besides giving the chief data in simpler form following the main summary of this experiment. Of course the results in all cases

are somewhat in error because of the number of offspring which die too early for identification; but the error is not considerable since all of the available evidence points to the conclusion that all of the different kinds of offspring are about equally viable. Inspection of this table shows no marked changes in the relative number of kinds of offspring from the first to the later broods in any of the generations in either the sexual or the asexual lines. This would corroborate the conclusion already reached from the numerous other experiments, namely that sex is in no way correlated with the age of the mother. It was of the greatest interest to find that the offspring of the  $F_3$  generation in the sexual line were no more likely to be males and sexual females than were the offspring of the  $F_3$  generation in the asexual line. It seems that if such selection were to have any definite effect that it would at least begin to show by the third generation. Yet it will be observed that in the second generation 33.3 per cent. of the offspring in the asexual line is male, and 32.7 per cent. is male in the sexual line; while in the third generation the corresponding percentages are 35 per cent. and 31.5 per cent. respectively. Of the stem mothers the percentage of males in the offspring is 23.5 per cent., the lowest of all. It seems to me however that the differences in no case are great enough to be of any significance in a species of animals which displays so much variation in so many respects as this one does.

The ratio of the asexual to the sexual females is seen to be 27.6 per cent.: 23.1 per cent. in the  $F_1$  generation; 19.1 per cent.: 15.3 per cent. in the  $F_2$  generation of the asexual line, 21.9 per cent.: 11.3 per cent. in the  $F_2$  generation of the sexual line; 20.7 per cent.: 17.6 per cent. in the  $F_3$  generation of the asexual line, and 28.2 per cent.: 12 per cent. in the  $F_3$  generation of the sexual line. The last ratio is striking as showing such a low percentage of sexual females in the sexual as compared to the asexual lines in the same generation.

It will be seen that if the percentages of the males in these five categories are averaged we get somewhat over 31 per cent., which is very much lower than that observed in experiment 2, which was 42 per cent. Since in the latter case the offspring considered numbered over 1,700, it may seem that we should be

justified in concluding that the percentage of males in the generations immediately following the stem mothers is normally low. Such a conclusion would be erroneous, since several experimenters have succeeded in maintaining cultures of *Simocephalus* and other genera indefinitely in the parthenogenetic phase. In all cases in my experiments where females were isolated and kept in small containers in the laboratory some of their broods were sure to contain sexual forms if the mothers lived to produce a reasonable number. In the experiment under discussion the sexual forms did not increase up to the sixth brood, after which so large a proportion of stem mothers died that it seemed best to close the experiment. However at this point experiment 5 is complementary since it is concerned with a large number of females which are of varying degrees of remoteness from stem mothers and we find them producing only about five per cent. of sexual forms. It seems quite clear that the remoteness of the generation from the stem mother bears no definite relation to the numerical ratio of male to female offspring or of sexual to asexual females.

As to the degree of sexuality of the sexual females concerned in this experiment I find no evidence that it is different from that observed in the former experiment. The number of ephippia produced can doubtless be taken as a crude index to the degree of sexuality of a female, since, as shown elsewhere, the number produced is dependent upon factors inherent in the female, fertilization of the ephippial egg having no relation whatever to the continuance of the sexual state. The number of ephippia cast by each sexual female in this experiment averages slightly less than two. I have found very few females which produce as many as five ephippia. Only three instances are recorded in all of my experiments. The production of three is common. Here as in all other cases observed, the sexual individuals passed the sexual state by the time they were about half to two thirds grown, and never returned to it once they had become asexual. The only observer so far as I am aware recording the contrary for *Simocephalus vetulus* is Issakòwitsch (1908). Moreover, ephippia which were barely noticeable would sometimes appear and be cast, asexuality coming on at once. Other individuals would develop their ephippia to half or two thirds the normal size and





become asexual. After the production of several ephippia a partial one may be developed as the female leaves the sexual state, or she may change over abruptly. The degrees of sexuality are infinite, since it is subject to continuous and not to integral variation.

Considering the stem mothers from the standpoint of size attained, viability, length of life, activity, etc., in the first few generations of offspring, in addition to the proportion of the kinds of offspring as to sex, and the degree of sexuality of the females, one is forced to conclude that stem mothers are not functionally at all unlike the females which are produced parthenogenetically, with the exception that not a single instance of the production of an ephippial egg by a stem mother has been noted. This last is in strict accordance with the findings of Grosvenor and Smith (1913) for the stem mothers of *Moina rectirostris*, and of most writers, though rarely instances of the contrary are noted, e.g., Scharfenberg (1911, p. 24).

The most important data are excerpted from the tabulated summary of experiment 3 and slightly rearranged in the following table for the convenience of the reader.

Kinds of Offspring.	Asexual Fe- males.	Sexual Fe- males.	Males.	Other Fe- males.	Sex Not Known.
Percentage of kinds of offspring (F <sub>1</sub> gen.) in the first 6 broods of 24 stem mothers. . . . .	27.6	23.1	23.5	15.4	11.3
Percentage of kinds of offspring (F <sub>2</sub> gen.) in the first 6 broods of 16 F <sub>1</sub> asexual females . . . . .	19.1	15.3	33.3	15.6	16.1
Percentage of kinds of offspring (F <sub>2</sub> gen.) in the first 6 broods of 16 F <sub>1</sub> sexual females after becoming parthenogenetic. . . . .	21.9	11.3	32.7	31.2	2.6
Percentage of kinds of offspring (F <sub>3</sub> gen.) in the first 6 broods of 12 F <sub>2</sub> asexual females . . . . .	20.7	17.6	35	21.6	5.1
Percentage of kinds of offspring (F <sub>3</sub> gen.) in the first 6 broods of 12 F <sub>2</sub> sexual females after becoming parthenogenetic. . . . .	28.2	12	31.5	19.1	9.2

Experiment 4. Temperature experiments with *Simocephalus* yield very indefinite results. Individuals do not thrive at a higher temperature than 28° C. Even at this temperature isolated individuals live only a short time. Several experiments designed to test the effect of high temperature were performed in the following manner. Specimens were taken from laboratory cultures and placed in glass containers having loose covers to

prevent excessive evaporation. In each of these experiments four containers were used, of about one pint capacity, two thirds full of water at room temperature when the *Daphnia* were introduced. Each vessel contained a sufficient quantity of green algae for 25 females. These containers were placed in an electric paraffin bath and the temperature maintained at 28° C. The first one or two broods produced under high temperatures were found to contain the usual proportions of males and the two kinds of females (see tabulated summary of experiment 3). These first broods were discarded. Thus all of the offspring in the tabulated results of one of these experiments given below, passed their entire ontogeny at a temperature of 28° C.

Dates on Which Offspring were Removed.	Total Number of Mothers Living on Dates Indicated.	Offspring.				
		Asexual Females.	Sexual Females.	Males.	Other Females.	Sex Not Known.
August 25.....	106	21	0	28	16	3
August 30.....	102	14	12	30	42	17
September 5.....	81	6	10	11	38	8
September 7.....	17	2	0	7	5	8
September 10.....	3	0	3	2	0	4
September 11.....	0					
Total number of kinds of offspring.....		43	25	78	101	41
Percentages.....		14.9	8.7	27.0	34.0	14.0
Total number of offspring.....						288

The most important point to be noted in these results is that all kinds of offspring continued to appear to the last. Although the percentages vary somewhat, they are within the limit of normal variation. Other experiments were performed to determine the effect of lower temperatures. All of the kinds of offspring were secured at a temperature of 14° C., but the general metabolism is so much lowered at this temperature that the individuals were too few to justify any conclusions as to the ratio of the sexes. Both low and high temperatures, since they lower the rate of metabolism, induce a decrease in the average size of the broods, in the number of broods, viability of the offspring, and shorten the lives of the females used in experimenting. In this connection it is of interest to note that females which were reproducing parthenogenetically have been obtained every

month in the year from shallow ponds near Gary, Ind. The offspring obtained in the winter months were asexual females. While no sexual females and males were obtained from eggs and embryos borne by females at the time of collecting from under the ice, the offspring thus secured were not sufficiently numerous to justify any statement as to the production of males and sexual females at this season. I am uncertain as to just how far these experimental results hold true in nature, but there seems to be no reason for considering temperature a vital factor in relation to the sex cycle of *Simocephalus vetulus*. As mentioned elsewhere, Grosvenor and Smith (1913) completely inhibited sexual forms in *Moina rectirostris* at a temperature of 28° C., but since they could not decide as to what factor their success was due it is probably safe to assume that they succeeded in spite of the high temperature, not because of it.

*Experiment 5.*—The following experiment was performed to discover what kinds of offspring are produced under natural conditions. It involves 51 females collected at various times from April 20 to May 25, in the vicinity of Northfield, Minn., from three widely separated permanent freshwater ponds. The sizes of the females ranged from small (hence young) to very large (hence old) individuals. The largest female among the lot was slightly over 4 mm. from the anterior margin of the head to the posterior end of the carapace and  $2\frac{5}{6}$  mm. in vertical measurement. There is no doubt that the age and size of *Simocephalus* correspond very closely. Though I know the pedigree and brood records of none of these 51 females it may be assumed on the basis of size that some, such as the smallest, had produced very few broods, while others, the larger ones, had produced very many, probably 15 or 20. Although the individuals were not isolated there was no crowding. They were placed in two-quart fruit jars, no jar containing more than six individuals. Jars containing several specimens were kept full. They were placed outside the laboratory windows where the sun would not strike them, being thus subject to the ever varying temperatures. Only water brought from the ponds where the females were collected was used. This was strained through a fine silk cloth which removed all metazoa, but allowed

the passage of small unicellular algæ. After the first few days a gradually thickening film of green algæ grew on the sides of the jars. To insure that slight accumulations of excreta and other waste might not affect them the water was changed every two days. No food was provided other than that which was suspended in the water and which passed through the fine strainer. They were thus subjected to the same culture medium, food and the same night and day temperatures, as they were in the natural environment. It was thus hoped to duplicate as nearly as possible the natural conditions of these ponds and to acquire positive evidence as to the kind of offspring being produced under natural conditions by testing only the first broods produced after the females were collected. It will be noted that the tabulated presentation of this experiment gives the dates of the collecting trips in the first column, the number of specimens secured on each trip in the second column, the dates on which all young were separated from their mothers and placed in other similar jars in the third column, the number of offspring removed on each date in the fourth, the number of offspring which proved to be female in the fifth, etc., Records were kept of more than one brood from the females collected on May 17, May 20 and May 25, and it is interesting to note that the fourth brood produced by the single female collected on May 17 consisted of 50 males and one sexual female. In calculating the percentages of kinds of offspring only those first removed were taken into consideration. Of the 524 offspring in this category none is positively known to be male and 98.8 per cent. are positively determined to consist of females. All of the first offspring of the 19 females collected on April 20, May 16 and May 23 were saved to test for sexuality. It is seen that 95.6 per cent. were asexual, unless possibly there were more than 7 sexual females in the brood of 77 produced by the single very large female collected on May 16. Most of this brood died very early and the mother died also without further progeny. Excluding this one female's offspring the percentage of asexual offspring of the other 18 females is over 99 per cent. No sexual forms, either male or female, were collected, but a few ephippial eggs were secured by skimming the surface of the ponds and these on dissection seemed to be

perfectly fresh, and could hardly have been unhatched eggs of the preceding season. That they were *Simocephalus* eggs is quite certain since the only other Daphnian of similar size in these ponds is *Daphnia pulex*, whose ephippia contain two eggs each.

That the practical freedom from sexual forms in this instance is due to external conditions is rendered absolutely certain by the fact that these same females when the jars were placed in the laboratory and the water no longer regularly changed, begun to produce males and sexual females, in just about the same proportions as in experiment 3 and 4, as nearly as could be judged from general observation.

TABULATED SUMMARY OF EXPERIMENT 5.

Date Collected.	Number of Mothers.	Date of Removal of Broods.	Number of Offspring.	Fe-males.	Males.	Asexual Fe-males.	Sexual Fe-males.	Sex Unknown.
April 20	4	1st April 28	33	33	0	33	0	0
May 6	11	1st May 8	45	45	0	?	?	0
May 9	6	1st May 11	15	15	0	?	?	0
May 16	1	1st May 17	77	77	0	?	7	?
May 17	1	1st May 20	4	?	?	?	?	4
		2d May 23	15	15	0	15	0	0
		3d May 25	45	45	0	45	0	0
		4th May 30	51	1	50	0	1	0
		5th June 3	34	34	0	?	?	0
May 20	6	1st May 23	?	?	?	?	?	?
		2d May 26	120	118	2	?	?	0
May 23	14	1st May 25	150	150	0	149	1	0
May 25	8	1st May 30	200	198	?	?	?	2
		2d June 3	101	97	1	?	?	3
Totals . . . . .	51		890	828	53	242	9	9
Totals in 1st broods . . . . .			524	518	0	182	8	6
Percentages of kinds of offspring in the 1st broods . . . . .			100	98.8	0	95.6	4.3	?

## V. EXPERIMENTS WITH EPHIPPIAL EGGS.

Authors are not agreed as to the factors involved in shortening the latent period of the ephippial egg. Experimentation along this line has yielded the most inconsistent results. I have repeated the experiments of Weismann and others of freezing the eggs for varying lengths of time with all but complete failure. Hence I shall give in detail the experiments I have performed in

my attempts to discover a means of ending the latent period, although in not a single instance have results been all that could be desired. In a considerable number of my experiments I have made special effort to reproduce the factors operating upon these eggs in nature, by a series of freezings, alternated with drying, placing the eggs in the sunlight, etc., but the results seem to indicate that I have neglected the one thing needful. Of one thing I am quite certain, namely, that the fact that the eggs of *Simocephalus vetulus* can be collected from the fresh-water ponds every month in the year must be taken into account in formulating any general conclusions as to the factors concerned with their development. Unless otherwise stated, eggs of *Simocephalus vetulus* were used in the following experiments. In experiments performed with eggs collected from the field there were usually several kinds present but those of *Simocephalus* predominate. My only successes have been with *Simocephalus* eggs which were produced in the laboratory.

*Experiment 1.*—Since the eggs that are laid in ponds are, as the ponds dry up, subjected to gradually increasing concentrations of whatever salts may be in solution in the pond water it occurred to me that this might be a potent factor in terminating the latent period. To test this I took numerous ephippial eggs from my laboratory cultures and placed them in a pint of pond water and allowed it to stand exposed in the laboratory in a wide stender dish. I placed a graduated scale on the side of the dish, dividing the water depth into ten equal spaces. When the water had lowered to each of the various levels by evaporation, I took out a number of the eggs and placed them in covered vessels. Continuing thus until the water in the open dish had all disappeared, I left the last lot in the open vessel until it was thoroughly dry. The same experiment was tried starting with low concentrations of NaCl, but the results were negative in all cases.

*Experiment 2.*—A large number of ephippial eggs were collected from the surface of the ice on temporary ponds near Gary, Ind., on March 12, 1914, and dried at once. On June 20, 1916, they were placed in water at room temperature and left. They were watched for several months until it seemed

certain that none would develop. On August 15 the experiment was discontinued. If freezing is the most important factor as some authors have believed, it certainly cannot be all sufficient, for this lot of eggs numbered several hundreds and they were frozen up in the ice for many weeks before they were transferred to water. At two later dates other lots were collected and treated similarly. The last was on March 21, when the ice had begun to thaw.

One lot of eggs taken from a laboratory culture in April were dried for three days, then placed in water at room temperature but did not hatch. After two months they were taken out and dried. Over two years later they were again transferred to water at room temperature without success. These experiments were performed at the University of Chicago, but before leaving there I secured great numbers of ephippial eggs by skimming the surface of the ponds near Gary, Ind., and at intervals of every few months thereafter for three years placed a number of these in water. None ever hatched. The eggs treated in this way were not counted though 1,000 would be a very conservative estimate.

*Experiment 3.*—This experiment was performed with ephippial eggs which had been recently produced in the laboratory cultures. They were subjected to very low concentrations of  $H_2SO_4$ , namely,  $M/100$ ,  $M/10$ ,  $M/5$ ,  $M/2$  and  $M/1$ , for 1, 2, 10, 24 and 72 hours. Three eggs were used for each separate set of conditions. The eggs were transferred to water and examined daily. Results were negative. A precisely similar experiment was then carried out with KOH in the place of the  $H_2SO_4$ , without success.

*Experiment 4.*—On January 15, 1914, 10 ephippial eggs were taken from a laboratory culture and dried for three hours, then kept in a freezing mixture for eight hours after which they were transferred to water at room temperature. None hatched.

On the same date 6 ephippial eggs were transferred directly from a laboratory culture to water which was kept at  $30^\circ C.$  for four weeks. Another lot was kept at  $28^\circ C.$  Results were negative in both cases.

On January 18, 1914, some ephippial eggs which had been



produced in the latter part of December, 1913, in laboratory cultures, were subjected to the following conditions: One lot of 7 was subjected to a freezing mixture for eight hours, one lot of 6 to a freezing mixture for eight hours on two successive days, and another lot of 6 were given the same treatment on three successive days. In each case the eggs were transferred from the freezing mixture to water at room temperature. The same kind of treatment was applied to large numbers of eggs which had been collected by scraping the surface of the ice on fresh water ponds near Gary, Ind., permitting the eggs to dry between the freezings. Others were given the same treatment and then dried for three months before being transferred to water at 14° C., but all to no avail.

*Experiment 5.*—During the course of my work I have made numerous attempts to induce development by clipping off the small ends of the ehippia with scissors or scalpel, by pricking the shell with a needle, and by dissecting it completely off. It is hardly to be supposed that such treatment would have any effect upon eggs that had never been dried, since they often have the ventral edges of the ehippia only lightly apposed, it being possible in some instances to look between them upon the egg inside, but conceivably desiccated eggs might be thus influenced. It is quite a simple matter to spread the valves and set the egg free. I have succeeded in three instances in placing ehippial eggs with the shells removed in the brood pouch of asexual females. This did not incite development.

On August 14, 1916, I dissected the shells from 18 ehippial eggs and pricked them with an extremely fine glass needle. In some cases I barely pierced the vitelline membrane, and in others pushed the needle about one third the way through the egg. The membrane is quite tough and in most cases is under considerable tension due to osmotic pressure. There is a quantity of liquid underlying the membrane and minute quantities of this can be seen to exude from the wound in all cases, and if the wound is made with too large a needle or too deeply the granular cytoplasm is extruded. Presumably such eggs are destroyed. Fourteen of these eggs seemed to have been successfully treated and were placed in a stender dish with enough

algæ to nourish any embryos that might appear. They were examined daily for some weeks and at intervals for two months. Some of the eggs degenerated early and others persisted as if no injury had been received. None had developed on October 10, and shortly thereafter no eggs could be found.

*Experiment 6.*—On January 15, 1916, two dozen eggs were taken from a laboratory culture which had been producing an abundance of them, and without being allowed to dry, were placed in a vessel where the sun would strike them for a few hours daily, for some weeks. They were then placed out of the sun. On August 16 I placed them on ice for 24 hours, and then left them until June, 1917. That they were in perfect condition at this time was shown by the fresh green color of the eggs on removal of the ephippia. They were held under water by being placed in a vial which was left lying on its side in a stender dish. None of these developed.

*Experiment 7.*—On June 30, 1916, a number of ephippial eggs of *Daphnia pulex* were taken from a laboratory culture and placed under water in the same manner as in the above experiment and left thus submerged until May 20, 1917. Dissection of the ephippia from several of these showed them to be in good condition at the end of this time, none having developed.

*Experiment 8.*—Although all of my carefully devised attempts to induce ephippial eggs to hatch by the application of chemical and mechanical stimuli, as well as the numerous repetitions of the freezing experiments of Weismann, have completely failed, I have succeeded in securing about 70 stem mothers from some *Simocephalus vetulus* eggs which had been given no special treatment. On June 27, 1916, I removed several hundred eggs from a culture which had produced great numbers of them and placed them in a large-mouthed 8 oz. bottle lying in a horizontal position in a one-gallon battery jar about one third full of water. A small amount of unicellular green algæ was added. Cypris of several species soon appeared in large numbers. On August 14 an embryo of *Simocephalus* appeared. I removed it in a little of the same water together with some of the algæ. It lived only three days. Fortunately others were to follow. On August 18 another appeared, and produced a brood of 7 females

on August 24. Eight more hatched on August 21, 7 more on August 25, 10 on August 26, 6 on August 30, 4 on September 3, 2 September 6, 1 September 20, and at more or less irregular intervals for several months thereafter others continued to appear, until nearly six dozen had been secured.

Two other instances deserve mention. Three days before setting up this experiment I had placed a number of eggs, produced from May 1, to June 26, in a jar in a similar manner and they remained there until December 12, when they suddenly began to hatch, but only a few appeared. I can suggest no explanation as to why they did not all hatch, or why the few that did should not have done so earlier as did most of those mentioned above. On August 9 some fresh eggs were placed on ice and left 36 hours and then placed under water in the manner described above. On December 27 two hatched. No more appeared although the experiment was left standing until June 1, 1917, *i.e.*, about 11 months.

Mortality among these stem mothers was found to be about the same as with other females. I succeeded in obtaining an average of a little over six broods from each of 24 of these stem mothers and have given their history in the interesting experiment (number 3) already described.

It should be stated that there were numerous other eggs in the culture from which the majority of the stem mothers was secured, which did not hatch. Some of the water from this culture was removed and other newly produced eggs placed in it. This experiment was left standing from August 30, 1916, until May 5, 1917. Since none of these developed we must infer that the kind of water in the battery jar containing the developing eggs, was not the responsible factor, though I have not the remotest idea what it may have been.

## VI. OBSERVATIONS ON PAIRING.

A female from a culture containing many ephippial females was isolated on July 19. By July 27 she had produced two female broods, one of 14 and another of 10. I placed the older brood with males. On August 1st I found that 14 ephippia had been produced, though 10 of them were empty, and all but one of

the females had become asexual. The one sexual female remained so until she had developed three ephippia. Then she developed a brood of 5 asexual females and died August 9. It was not surprising to find so large a percentage of empty ephippia appearing in the presence of males since the same thing occurs in general cultures, though I had thought it might, in the latter case, be due to too small a percentage of males. That this explanation is erroneous is shown by this and the following experiments.

Several of the female broods obtained from the two females isolated in isolation experiment number 1, were placed with numerous males and examined daily. On July 1 more males were added since I noticed several incipient ephippia appearing. On July 30 the culture numbered about 150 individuals, and only two ephippia had fully developed, one of these being empty. By August 6 there had been developed 14 ephippia, most of them empty. The percentage of ephippial females thus remained less than 10 per cent., so low that it is quite obvious that the presence of males could not be the factor responsible for their appearance, since larger proportions of sexual females often occur in females which are segregated from the males. Not all of the ephippia reached full size, though most of them did. There was a dearth of males at no time during this experiment, yet less than half of the fully developed ephippia contained fertilized eggs. The fourth brood of female number 18 of isolation experiment 3, consisted of 7 females. These when very young were placed with 10 males. All seven, however, proved to be asexual, although some of their offspring developed into sexual females in the absence of males.

The 20 females of the fifth brood of female number 38, of isolation experiment 2, were divided into two lots, 10 being kept with males and 10 without. Of the 10 with males, 9 developed ephippia, a few of which contained eggs when the molt was cast. These eggs, however all degenerated, so presumably they had not been fertilized. The males had all died at this time. Six of these females became asexual after producing the first ephippium. I placed some more males with the remaining four sexual females and was fortunate enough to observe two matings of

the same female within two hours. I saw no spermatozoa lost in these instances as I have in several cases observed later, and I have no doubt that the one normal ephippial egg which I found later was the result of this pairing.

Of the 10 without males, one died early, 5 developed ephippia, and 4 became asexual at once. The 5 sexual females developed in all 16 ephippia, which is more, and more per individual, than was produced by those kept with males. Males appeared in some of the broods of the asexual females, but most of the ephippia were produced before they appeared, and there was only one fertilized egg. The sexual females all became asexual before death.

The 35 females of the sixth brood of this same female number 38, were also saved. The one male of the brood died at the age of four days. Of the 16 ephippia produced by the members of this brood one contained an egg. Since the egg did not degenerate it must have been fertilized. Thus we have proof of the early functioning of the males. They are shorter lived than the females, or at least that has been the case with individuals isolated. It will be noted that this brood contained three kinds of individuals, namely asexual females, sexual females, and a male. My records contain numerous instances of this kind.

All of the females of broods 4, 5, 8 and 11 of female number 41 of isolation experiment 3, were placed with males and a complete record kept of the results. The fourth brood consisted of 7 males and 7 females, all sexual. In all 17 ephippia were cast by these 7 females. Two of them then died and the remainder became asexual. In spite of the presence of the males 15 out of these 17 ephippia contained no egg. The fifth brood consisted of 5 males and two sexual females. These were reared together. Each female produced a fertilized egg and one of them an empty ephippium afterward, this one then dying. The other became asexual though the males were continually present. The eighth brood consisted of 20 females. A number of males were placed with these. Five of the females died early, and none of the rest developed even an incipient ephippium. The eleventh brood consisted of 3 females, 2 being sexual and 1 asexual. The

asexual one was discarded. These two females whose mother was asexual were reared under conditions which were as nearly alike as they could possibly be made and yet have them in two separate vessels, gave rise to diverse offspring. An equal number of males was placed with each. The history of one was: an empty ephippium, 4 males, 4 sexual and 2 asexual females, an undetermined brood, death. The history of the other was: an empty ephippium, a fertilized egg, a fertilized egg, 3 sexual and 3 asexual females, 1 sexual and 4 asexual females, death. Each produces an empty ephippium at first, but the former then produces a brood of 4 males, and the latter another ephippium, this time containing a fertilized egg. Both later produce both kinds of females. Several other experiments of this same kind gave similar results. I wish to point out here only one conclusion based on these experiments, namely, that all afford evidence that the reason for the production of male broods is not the production of ephippial eggs which were not fertilized because of the absence of males, for in these experiments the onset of male brood production has been observed many times in the females which have passed from the sexual state and begun the production of males, in the presence of other males.

The method of copulation is interesting. This was observed in several instances. The sixth brood of female number 44, of isolation experiment 2, consisted of 8 sexual females and 1 asexual female. When the first ephippia of these 8 sexual females were in their incipiency I introduced 5 males and watched them almost continuously, removing them when I could not give them attention. When the females were about 6 days old the ephippia were well developed and the ephippial eggs seemed to be ready to be extruded into the brood pouch. It was at this time that the monotony of proceeding was relieved. On being placed in the vessel with the females the males at once became intensely excited. This lasted nearly an hour. After several abortive attempts at pairing one pair mated. The male opened his carapace slightly and clasped the antero-ventral margin of one side of that of the female. He then bent his abdomen ventrad, so that it extended between the ventral margins of the female's carapace, ventral to her posterior appendages. In

this position he waved his abdomen about for a few seconds, then straightening it, he ejected the spermatozoa in two forcible streams, which, like puffs of smoke, spread abruptly when their force was spent. They seemed to me to be all swept away by the exhalant respiratory current of the female. The excitement of the males was now abated, and in about fifteen minutes I removed the males. Two hours later I introduced the males again and secured a second mating. In this instance the male ejected the spermatozoa just anterior to the posterior end of the abdomen of the female, and posterior to her last pair of appendages. Again I saw some of the sperm cells swept away, but very few. I am now convinced that the first case described was abortive. Other observations were made later and in some cases no part of the mass of spermatozoa was lost, though no case was observed in which the sperm cells were not ejected freely into the respiratory chamber as described, the only point of contact being the ventral margins of the male's carapace with the antero-ventral margin of one side of that of the female. The ephippial egg is laid within about an hour, and in no case observed more than two hours, after the pairing. During the period of œstruation the female doubtless produces some chemical substance which passes out in her exhalant respiratory current, since it is by passing through this that the males are made aware of the females' readiness to mate.

I have made oft repeated attempts to observe the copulation process from the lateral aspect. Several devices, such as a trough made of cover glasses and a slide, in which the specimens were placed, yielded only failures. The males do not always attach to the same side of the female's carapace. In case the male attaches himself to the left side of the female's carapace, the spermatozoa are ejected near that side of the respiratory chamber and pass to the left of her abdomen to the brood pouch, seemingly being driven dorsad by the action of her last pair of appendages. In one such case observed the ephippial egg was developed in the right ovary. In order to enter the oviduct the spermatozoa would have to pass across the brood pouch to the right side. The sperm cells are immotile. Though I have not yet been able to see them after they have been trans-



ferred to the brood pouch, I am convinced that they are retained in it and that fertilization takes place after the egg is laid.

#### VII. GENERAL DISCUSSION.

1. What relation does the presence of the males bear to the origin and prolongation of the duration of the sexual state in the females?

One gets the impression from the literature that the ephippial egg and ephippium are completely developed only if fecundation occurs. Perhaps enough data have already been given in the above discussion of the observations on pairing, of the effect of the presence of males in prolonging the duration of the sexual state of the females to convince any one that it is nil, though more evidence is at hand. I wish to add here that I have observed some hundreds of instances of embryo females developing through the stage at which they would become sexual, if at all, which never evinced the slightest evidence of sexuality. In a great many instances these were in the same containers with sister females which attained a high degree of sexuality. The males used in some of these experiments were related to the females, but in most instances they were not. It is evident that the ephippial egg and the ephippium are both developed to within one or two hours of the time of extrusion of the egg into the brood pouch, in complete independence of the male, the only definite correlations of these processes with the presence of males to be noted, being that the egg is usually not laid in the absence of fecundation, the exceptions being, as I should judge, about 1 per cent. to 5 per cent. I have observed about half a dozen instances.

2. In the same environment are individuals which pass through the sexual phase any more likely to give rise to males and sexual females than are individuals which are parthenogenetic from the beginning of their reproductive period?

Since the twenty-fourth female in isolation experiment 3 gave rise to pure broods of males, pure broods of sexual females, pure broods of asexual females, to mixed broods of males and females, and to pure broods of females of diverse sexuality, thus compassing the whole range of possibility as to kinds of offspring, she was presumably normal, hence I used some of her sexual

offspring with which to test this question. I selected 12 of the sexual females of broods 12 and 13. Each of these 12, after having produced one empty ephippium at the age of 11 days, became asexual. Two of the 12 died. In two other cases the first brood died too early for identification, but the first determinable broods of the 10 that lived were as follows:

1.	6 females,	1 sexual,	3 asexual,	and 2 undetermined.					
2.	7	"	2	"	3	"	"	2	"
3.	3	"	0	"	2	"	"	2	"
4.	3	"	0	"	3	"			
5.	2	"	0	"	2	"			
6.	10	"	4	"	6	"			
7.	6	"	1	"	5	"			
8.	3	"	1	"	2	"			
9.	12	"	1	"	11	"			
10.	6 males								

Thus in these first broods of the 10 sexual mothers only about 22 per cent. of the females are sexual, and about 12 per cent. of the offspring is male. All of the subsequent broods of these 10 mothers were also saved and identified. It was found that the percentage of males was slightly over 8 per cent., a decrease from that shown in the broods immediately following the ephippia. This can be of no special significance however since the percentage in that case is very much lower than one usually finds even in the offspring of asexual mothers, as noted in other experiments. As we have already seen, somewhat over 40 per cent. of the offspring, totaling over 1,700, of the 45 females concerned in isolation experiment number 2, is male. About 33 per cent. of the broods of these 10 sexual mothers are mixed as to sexuality, which is about normal. It will be recalled also that the percentage of males and sexual females in the sexual line of offspring from the stem mothers, (see isolation experiment 3) is actually slightly less than in the asexual line. It thus seems certain that the production of ephippial eggs at the beginning of the reproductive period, whether they are fertilized or not, has no influence on the subsequent offspring.

3. Is the age of the mother correlated in any way with the kind of offspring?

The twenty-fourth female in isolation experiment 2, gave as

her first two broods, pure male offspring. These were followed by three pure broods of females, and then a brood of 15 males. The seventh brood was a mixed brood. Her last 7 broods, totaling 66, were all females. The sexual and asexual females in these last broods occurred in about equal number, namely, 30 sexual, and 36 asexual. Numberless such instances might be cited to show that males often appear in the first broods. The summary of the data in isolation experiment 2 shows that the first broods were pure male in 16 instances, pure female in 26 instances, mixed in 2 instances, and undetermined in 1; while the last broods were pure male in 11 instances, pure female in 28, and mixed in 6. The tabulated results in isolation experiment 3 are also in accord with the data just given and are, I believe, quite conclusive on this point. It will be noted that there is no marked increase or decrease in the number of males, sexual females, or of asexual females, from the first of the sixth broods, inclusive, in the descendants of the 24 stem mothers, for the three generations, in either the sexual or asexual lines. When the first broods of newly collected old and young females are compared no difference in kind is noted, as shown in experiment 5. When these same individuals are kept in small containers in the laboratory, no matter if the food supply is abundant, if the water is not changed often, sexual forms appear in the offspring of young and old alike, and in like proportions. These facts point to the conclusion that there is no correlation between the age of the mother and the kind of offspring.

4. Do mixed broods indicate a transitional stage from male producing to female producing and vice versa?

The summary of isolation experiment 2 shows 9 instances of the production of mixed broods which are preceded and followed by broods of the same sex, while there are but four cases in which mixed broods are preceded and followed by broods of different sex. There are in this same experiment two cases in which the first brood was a mixed brood, and 6 cases in which the last brood was mixed. Quite often one mixed brood follows another. I have found no evidence in favor of the view suggested by this question, the occurrence of mixed broods in these experiments being entirely capricious.

5. What relation has sexuality to the duration and senescence of the laboratory cultures?

The fact that my cultures which have produced sexual females and males have always passed sooner or later into a more or less non-productive phase and did not die out provided that conditions were not so severe as to terminate them, is well deserving of consideration, in view of the fact that one so often reads in Daphnian literature of cultures becoming sexual and being thereby terminated. During the five years I have worked on *Simocephalus* I have run scores of cultures and I have never had an instance of a pure sexual culture except those set up by selecting males and sexual females from other cultures. At the most such cultures remain purely sexual only a few days, when with the passing of some of the sexual females into the asexual phase, broods invariably appear containing asexual females. I have no record of a large pure brood of sexual females, and no record of a female which has consistently produced pure sexual small broods. Broods containing sexual females may be produced by any female no matter what her pedigree has been. Naturally if a large percentage of the females in a culture are passing through the sexual phase, so much of their immediate productivity is sacrificed, but since the number of ephippial eggs produced rarely exceeds 4, and is usually only 1 or 2, and always being produced within the first 10 to 25 days of their lives, every sexual female devotes the most of her life to asexual reproduction. Old cultures, in which there has been much accumulation of excreta, run down, the size of the broods decreases to from 1 to 5, many individuals die before maturity, and the culture dies out finally; but so far as I am able to see, this neither causes nor results from the production of ephippial eggs.

It is of interest to consider what happens in laboratory cultures which are set up and allowed to run their natural course unhindered. With each female producing a brood of from 3 to 35 every 40 or 50 hours, as is always the case in a culture containing an abundance of fresh green algæ and no accumulation of excreta, any culture, even though started with a single individual, will become overstocked in a few weeks, and the increase in numbers has to stop, the development of eggs in the ovaries and the

growth of the embryos become retarded, the culture remains nearly at a standstill for some weeks, and if the food supply is exhausted will finally die out, though it has been my experience that they usually linger on indefinitely. By the time the culture has become overstocked ephippial eggs have appeared in considerable numbers, and after having passed the state of equilibrium, when the rate of reproduction has rather abruptly ceased, for some weeks just about but not quite equaling the death rate, the accumulation of ephippial eggs may be taken as proof that the culture has passed through a sexual phase, which in turn is likely to be erroneously considered the cause of the decline of the culture. And now with reproduction at its lowest ebb, and all of the sexual females having passed into the asexual state, as they all do, the observer is naturally likely to believe that the culture has passed into the asexual phase, since all of the sexual members of the culture have yet to live the asexual phase of their lives. Thus there will be an indefinite period when nearly every member in the culture will be asexual. It must be remembered that at this critical period, assuming the ordinary proportion of males, reproduction must be cut down to somewhat less than two during the life time, which is a few weeks or months, of each female. Considering that 25 per cent. of the females are sexual (see table, isolation experiment 3), and that each sexual female is actually carrying an ephippium one-fifth of her life time, even assuming that to be but 14 days it is obvious that only 5 per cent. of the females will be carrying ephippia at any given time. I believe the life time in a large culture is in reality much longer than I have assumed, and 25 per cent. far too great, and that it is safe to say that in many instances the ratio of females actually carrying ephippia at a given time is as low as 2 per cent. If it should happen that these few should cast their ephippia at the same time, for a day or so thereafter one could find a sexual individual only by a tedious hunt with the microscope.

On the other hand, when reproduction proceeds apace, starting with 10 females let us say, each producing only 6 broods, with an average of 1 and  $\frac{3}{10}$  sexual females to a brood, (as was the case in isolation experiment 3), we should have 78 ephippial

females in the first generation ( $10 \times 6 \times 1.3 = 78$ ); and supposing there were in all only 5 females to a brood, (see table of same experiment), we should have 300 females to mother the  $F_2$  generation, which should yield 23,400 ephippial females, out of a total of 90,000. Of course in practice, laboratory cultures are overstocked long before all of the second generation has appeared, and in fact reproduction has practically ceased by the time 100 to 200 ephippia have appeared. The accumulation of the ephippia makes the culture seem to be in the sexual phase. The variation in the number of kinds of offspring produced may depend to some extent upon the extreme prolificacy and consequent mortality in laboratory cultures, since the assumption of ever so slight a difference in the susceptibility of embryos to the great variety of adverse conditions met with in laboratory cultures, would be sufficient to account for much of the seeming variation. One may sometimes find several hundred ephippia in a laboratory culture in which there are very few males. In one such instance I collected 200 ephippia, only 2 of them containing eggs. Four explanations are possible: they may have hatched, which is very unlikely, there may have been too few males to fertilize them, mating may have been inhibited by some unfavorable cultural condition, or, there may have been abnormal pairing such as I have described in the section on pairing, in which case the spermatozoa not being retained, the eggs would degenerate. I have had a few small cultures in which for a short time males were very numerous and sexual females almost absent, but such instances are rare.

The following is typical of my experience with *Simocephalus*. I isolated a female on July 1, 1913, and kept her in lake water from lake Michigan. This was changed daily. She had nothing to eat except what she could get from the lake water. This seemed barely sufficient since reproduction proceeded very slowly. On August 1 I placed 6 of her descendants in a rich alga culture. Here they multiplied very rapidly. By August 13 there were 50 ephippial eggs floating on the surface, and males were also present in the offspring. This culture continued to produce asexual forms for some months, when the alga gradually died out individuals in the culture became less numerous, reproduction



having practically ceased. Another culture was established from this by selecting a few specimens at random and giving them a fresh supply of algæ. It ran through a similar period of great productivity, about the same number of sexual forms occurring, and declined as in the first instance. A third fresh culture was stocked by a single specimen from the last. It likewise gave rise to a great profusion of Daphnians, sexual forms appearing in considerable numbers. Unfortunately I did not keep a record of the time it took in the last two instances for the sexual forms to appear; but, as shown above, the first culture, started August 1, produced 50 ehippial eggs by August 13, and that in the presence of an enormous quantity of green algæ, the container being a three gallon jar which had previously been used for *Paramecia*. This *Paramecium* culture had been started with boiled wheat as the source of nutrition and had been permitted to stand for nearly a year. The *Paramecia* had disappeared and green algæ had developed in immense quantities on the side of the jar away from the light. Dearth of food could not have been a factor in inducing sexuality in this culture. I have already shown in isolation experiment 2 that sexuality comes on in spite of an abundance of food, in small vessels containing only a single individual female. The study of general cultures leads to conclusions which are in agreement with results obtained by a study of isolated females, namely, that onset of sexuality is independent of food shortage, and suggests that it is related to accumulations of certain excretions, which become critical in their effect upon the kind of offspring surprisingly early in *Simocephalus* cultures which are allowed to run their natural course unhindered. Production of ehippial eggs reaches its highest level about the time a general culture is over stocked and the rate of reproduction has begun to decrease, passing to lower levels as the food supply decreases, as is readily demonstrable if one will but take the trouble to remove them as they appear in such a culture, recording the daily output.

Of all the females isolated for study and kept in small containers in the laboratory, including several hundred selected at random and as many more whose pedigree was known for several generations, also 60 stem mothers and all of their female offspring that



lived, for three generations, the only instances in which both males and females did not appear in some of the broods were those in which they were very few, *i.e.*, in which the isolated females died early. The same was true of sexual females. They always appeared in some of the broods unless they were too few. Pure broods of sexual females were very rare and they have never predominated in any of my cultures no matter how severe the conditions were. Though I have not carried out a definite experiment to determine how many generations a culture may be carried through the sexual females alone (except in isolation experiment 3, where distinct lines were carried to the third generation) I have not the slightest reason to suppose that they may not be carried on indefinitely, since each female, whether sexual or asexual, gives rise to both sexual and asexual females, provided only that the food supply and other cultural conditions have proper attention. I am convinced that much of the confusion in the literature on the relation of sexuality to the senescence of cultures is due simply to the extreme prolificacy of the Daphnians and the complex phenomena resulting therefrom. Sexual phase and asexual phase may well be applied to individuals, and asexual phase to cultures, but in *Simocephalus vetulus* cultures never become wholly sexual, only partially so, since in the most sexual of cultures the offspring of a given female are always mostly asexual, and the sexual females pass most of their lives in the asexual state. Finally let us recall that at the extremely modest rate of production of ephippial eggs assumed in our calculations above, that starting with 10 females we should have 23,400 ephippia at the end of the second generation, whereas, as a matter of fact the most sexual of cultures of *Simocephalus vetulus* produces only a few during a course of many months. Hence it is folly to consider the onset of sexuality causally related to senescence of cultures in this species.

6. Does *Simocephalus vetulus* depend upon external factors to call into expression maleness, sexuality in the females, and parthenogenesis?

With respect to this point the results of Grosvenor and Smith (1913), Banta (1914), and Agar (1914b) are particularly instructive. They succeeded in completely inhibiting sexual forms in

several genera of Cladocerans for an indefinite number of generations. Their conclusions are more fully discussed under the head of literature review.

In my earlier experiments there are numerous instances of the production of all three kinds of individuals, namely, males, sexual females and asexual females, by the same mother. Less common is the production of only two kinds of offspring during the life time of a female. In these experiments I have no record of a female producing only one kind of offspring except those whose progeny are very limited because of the early death of the mother, and in recent experiments, those which produce only asexual females, this last being of much importance. Rarest of all yet quite often met with is the production of all three kinds of offspring in the same brood. Female number 45 of isolation experiment 2 has one such brood containing only three embryos. Broods of this type are usually larger. The asexual females have nearly always outnumbered the sexual females and males in mixed broods, as they usually do in the sum of the broods of a given female.

Similar proportions of the kinds of offspring may be secured under a great variety of kinds of environment. In the most highly sexual cultures many pure asexual females are always present, and the sexual females remain so only for a brief period in early life. While these facts do not prove that the environment has nothing to do with the ratio of kinds of offspring, they would seem to indicate that it is not the determinative factor in the nexus of causes that are responsible for the expression of the species in all of its forms, regardless of internal factors.

## VIII. SUMMARY.

### I. *Life History.*

1. The offspring of any female of *Simocephalus vetulus* in the asexual phase may consist of any one or all three of the following kinds of individuals:

(a) Sexual females, which produce a series of from one to seldom more than six ephippial eggs early in life, then become parthenogenetic and so remain, being then indistinguishable from other parthenogenetic females. The final ephippium is

often only partially developed, showing the sexual capacity to be gradually lost.

(b) Parthenogenetic females, which display no tendency to produce ephippial eggs.

(c) Males.

The kinds of offspring occur in no definite order, but their character is probably determined at birth, and not by subsequent conditions. (See summaries of isolation experiments 2 and 3.) Eggs which will develop into males, into highly sexually females, and parthenogenetic females, often arise in the same female and not infrequently at the same time, either early or late in her reproductive period, whether or not she has passed through the sexual state herself.

2. The sequence of the generations is very indefinite:

(a) The stem mother is functionally like the females produced parthenogenetically, except that she probably never gives rise to ephippial eggs. (However see Sharfenberg, 1911, p. 24.) There is not even an approximately definite number of generations in the cycle from one stem mother to another. It may be one or many.

(b) The remoteness of the generations from the stem mother bears no definite relation to the percentage of males produced, the ratio of sexual to parthenogenetic females, or to the duration of the sexual state when present.

(c) The sexual state is probably determined in the ovary of the preceding generation. There are almost certainly predisposing factors in the environment but it is not certainly known what they are. Food or lack of food does not offer a sufficient explanation. Sexual females and males tend to arise at the same time, presumably in response to the same environmental complex.

(d) Cultures are indefinitely viable parthenogenetically. The species will express itself in all of its forms under a great variety of conditions. Under certain conditions sexual forms are completely inhibited. Parthenogenesis cannot be completely inhibited in cultures or even in individual females. Thus cultures of *Simocephalus vetulus* can never be terminated merely because of the onset of sexuality.

(e) The production of mixed broods is not to be interpreted

as evidence that the female producing them is undergoing transition from male producing to female producing or vice versa.

3. The production of ephippia and ephippial eggs are related but not causally, both being dependent upon common internal factors. The introduction of males into a culture does not induce the production of ephippial eggs, nor does their presence have any relation to the prolongation of the sexual state when it has once appeared.

## II. *Breeding Habits.*

Sexual attraction is limited not only to sexual females in the sexual state, but is confined to a limited period of a few hours before the ephippial egg is laid. It seems to be due to some kind of substance omitted by the female and borne by her exhalant respiratory current, where it is detected through a chemical sense by the male. Fertilization seems to take place in the brood pouch after the egg is laid. The presence of the spermatozoa in the brood chamber is probably the stimulus for its extrusion. In the absence of fertilization the ephippial eggs are usually resorbed in the ovary, or, if laid they undergo degeneration in the ephippium within one or two days.

## III. *Theoretical.*

The immediate significance of the ephippial egg is as a stage in the life cycle resistant to adverse conditions, and not in the stem mother hatching from it, since her offspring are in no way unique. A more remote but more fundamental significance, held in common of course, with all fertilized eggs and zygotes, is that it provides for the permanent liability of the species through amphimixis. In view of the great prolificacy of the species in regard to all of its forms, and the almost universally concomitant occurrence of males and sexual females, the development of only 1 per cent. of the ephippial eggs would be quite sufficient to secure to the species all of the benefits to be derived from their two functions. It seems probable that there is a very great inherent variability in the capacity for development of the ephippial eggs in a state of nature, and the lack of uniformity of results obtained by the various investigators in their attempts to shorten the latent period may be explainable on that basis.

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